275. *Natural Glycosides.* Part V. Ruberythric Acid.*

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As a result of the investigations of Rochleder, Schunck, Liebermann, and Bergami, and Schunck and Marchlewski (for references, see Perkin and Everest, "Natural Organic Colouring Matters," 1918, p. 35), it has been considered that ruberythric acid is a bioside of alizarin, which on hydrolysis yields two molecules of glucose. Zemplén and Müller (*Ber.*, 1929, **62**, 2107) synthesised a β-gentiobioside and a β-cellobioside of alizarin but, although the melting points of the cellobioside and its octa-acetate are very close to those of ruberythric acid and its acetate, these authors expressed the view that neither compound is identical with the natural bioside.

The syntheses of alizarin β-gentiobioside and β-cellobioside have been repeated, and in the former compound the biose residue is shown to be attached at the 2-position, since on methylation the hepta-acetate gave rise to a *methyl* ether, which on hydrolysis yielded 1-*O*-methylalizarin. By analogy, therefore, it seems reasonably certain that the synthetical β-cellobioside and the octa-acetyl β-maltoside (Robertson, J., 1930, 1136) are similarly constituted. This view is also supported by the fact that in the synthetical alizarin β-glucoside the glucose residue is in the 2-position (Robertson, *loc. cit.*). A direct comparison has shown that ruberythric acid † is not identical with either alizarin 2-β-gentiobioside

* The general term "glycoside" has been substituted for "glucoside," used in the titles of Parts I—IV, in view of an early extension of these studies to more complex members of the series.

† We are indebted to Professor R. Robinson, F.R.S., for a small specimen of ruberythric acid from the Schunck collection, the University of Manchester. The *O*-acetyl derivative, m. p. 230°, was prepared according to the directions of Liebermann and Bergami (*loc. cit.*), who consider it to be an octa-acetate.

or alizarin 2-β-cellobioside; this result was confirmed by a comparison of the respective *O*-acetyl derivatives. Further, the compound is not a β-maltoside, since its *O*-acetyl derivative is not identical with the synthetical octa-acetate of alizarin 2-β-maltoside.

Schunck (*loc. cit.*) has shown that ruberythric acid is hydrolysed by the enzyme erythrozym present in madder, but the behaviour of the compound towards emulsin does not appear to have been examined. We have found that ruberythric acid is hydrolysed by emulsin (supplied by the British Drug Houses, Ltd.), indicating that the glycoside linking at the aglucone residue is of the normal β-type. Though we were able to identify the resulting alizarin, lack of material prevented a detailed examination of the sugar residue. Application of the phloroglucinol and the orcinol reaction for a pentose to the aqueous solution obtained by either enzyme or acid hydrolysis gave a positive reaction, indicating the presence of a pentose residue in the glycoside, and in consequence it is probable that ruberythric acid is a pentosido-β-glucoside of the primeveroside or vicianoside type [$C_{14}H_7O_3$–O(β)–$C_6H_{10}O_4$–O–$C_5H_9O_4$]. This view is not inconsistent with the somewhat divergent analytical results which have been obtained for the compound, *e.g.*, the analytical figures given by Liebermann and Bergami (*loc. cit.*) for ruberythric acid and its acetate are in agreement with the calculated results for a monohydrate of a pentosido-glucoside and its hepta-acetate respectively (the presence of rhamnose is not excluded). On account of the comparatively wide distribution of primeverosides (cf. Armstrong and Armstrong, "The Glycosides," 1931, p. 76) the *hepta-acetate* of the 2-β-primeveroside of alizarin has been synthesised, but the compound is not identical with the acetate of ruberythric acid.

In view of the fact that ruberythric acid dissolves in alkalis forming red-coloured salts, the compound is undoubtedly a bioside and not a diglycoside (cf. Müller, *Ber.*, 1929, **62**, 2793), but there is no evidence to show at which hydroxyl group the biose is attached.

Experimental.

O-Hepta-acetyl-β-gentiobioside of 1-O-*Methylalizarin.*—The hepta-acetate of alizarin β-gentiobioside was prepared by the following modification of Zemplén and Müller's method (*loc. cit.*). The mixture obtained from the interaction of alizarin (2·5 g.), hepta-acetyl α-gentiobiosidyl bromide (6 g.), silver oxide (5 g.), and quinoline (10 c.c.) was dissolved in boiling acetic acid (100 c.c.), and the solution treated with charcoal, filtered, and gradually added to warm water (600 c.c. at 55—60°). The precipitated hepta-acetate was collected, washed, and crystallised from acetic acid–alcohol, m. p. 260° (Zemplén and Müller give m. p. 258°). Acetylation of this compound with acetic anhydride and sodium acetate at 120° for ¾ hour gave rise to the octa-acetate, which separated from acetic acid–alcohol in pale lemon-yellow needles, m. p. 236° (Zemplén and Müller give m. p. 232°). Mixed with the acetate of ruberythric acid, it melted at 208—210°.

A mixture of the above hepta-acetate (0·6 g.), methyl iodide (6 c.c.), silver oxide (6 g.), and acetone (100 c.c.) was refluxed for 8 hours; after 5 hours, further quantities of oxide (3 g.) and methyl iodide (4 c.c.) were added. A test with cold alcoholic alkali then showed that the methylation was complete, and after the addition of warm acetone (150 c.c.) the silver salts were removed by filtration and extracted with a further quantity of boiling acetone (200 c.c.). Evaporation of the combined solutions under diminished pressure left the *ether* as a crystalline residue. Recrystallised from methyl alcohol–acetic acid, it formed pale yellow needles, m. p. 240°, sparingly soluble in alcohol or acetone and readily soluble in warm acetic acid (Found: C, 56·1; H, 5·3. $C_{41}H_{44}O_{21}$ requires C, 56·4; H, 5·1%). To a solution of this ether (0·5 g.) in hot acetic acid (20 c.c. at 90°), concentrated hydrochloric acid (7·5 c.c.) and then methyl alcohol (20 c.c.) were added. The solid, which separated immediately, redissolved on warming, and the solution was gently refluxed for 1·5 hours and diluted with water (100 c.c.). 14 Hours later, 1-*O*-methylalizarin was collected and recrystallised from warm methyl alcohol, forming orange-yellow needles, m. p. 179°, identical with an authentic specimen. Acetylation of this compound with acetic anhydride and pyridine on the water-bath for 1 hour gave the acetate, which separated from methyl alcohol in pale yellow needles, m. p. 212°, not depressed on admixture with an authentic specimen.

Alizarin β-Gentiobioside.—5% Aqueous sodium hydroxide (60 c.c.) was carefully added to a suspension of the hepta-acetate (1·2 g.) in warm methyl alcohol (100 c.c.), and the resulting cherry-red solution kept at 60° for 20 minutes and acidified with acetic acid. Addition of an

excess of aqueous basic lead acetate precipitated the bright red lead salt of the bioside, which was collected, washed with alcohol, suspended in hot alcohol (250 c.c.), and decomposed by means of hydrogen sulphide. The boiling solution was filtered from lead sulphide, and on cooling deposited the bioside in thick, orange prisms (0·45 g.), m. p. 178—180° after having been recrystallised from alcohol and dried. Crystallised from warm water, it formed a *hexahydrate* in elongated, hexagonal, orange-yellow prisms, m. p. 96—98° (Found, in air-dried material: C, 46·6; H, 5·9; H_2O, 15·9. $C_{26}H_{28}O_{14},6H_2O$ requires C, 46·6; H, 6·0; H_2O, 16·1%. Found, in material dried in vacuum over phosphoric oxide for 48 hours and then at 120° for 2 hours: C, 55·0; H, 5·2. $C_{26}H_{28}O_{14}$ requires C, 55·3; H, 5·0%). Mixed with ruberythric acid, the anhydrous gentiobioside began to melt at about 150—155°.

Alizarin β-*Cellobioside.*—The hepta-acetate was prepared by the method used for the gentiobioside and had m. p. 254—255° after recrystallisation from ethyl formate (Zemplén and Müller give m. p. 249°). Treatment of this derivative with acetic anhydride and pyridine on the water-bath gave rise to the octa-acetate, which separated from acetic acid–alcohol in diamond-shaped plates; on heating, these melted at 143—145°, became solid at 170—180°, and then remelted at 224—225° Zemplén and Müller give m. p. 228—229°). The compound behaved in the same manner after repeated crystallisation, and was not identical with the acetate of ruberythric acid.

Deacetylation of the hepta-acetate (0·5 g.) was effected in methyl alcohol (50 c.c.) with 5% aqueous sodium hydroxide (60 c.c.), and the cellobioside purified by way of the lead salt as described for the gentiobioside. Crystallised from alcohol or dilute acetic acid, it formed slender yellow needles, m. p. 260° (Found, in material dried at 120—125°: C, 54·9; H, 5·3%) (Zemplén and Müller give m. p. 253°). Mixed with ruberythric acid, it melted at 240—245°.

Hepta-acetyl β-*Primeveroside of Alizarin.*—Hepta-acetyl primeverose was synthesised by the following modification of Helferich and Rauch's method (*Annalen*, 1927, **455**, 168). Silver oxide (2·5 g.) was added to a solution of 1 : 2 : 3 : 4-tetra-acetyl glucose (3 g.) and triacetyl α-xylosidyl bromide (Levene and Sobotka, *J. Biol. Chem.*, 1925, **65**, 465) (6 g.) in warm benzene (50 c.c. at 35°), and the mixture vigorously shaken for ½ hour and then refluxed for 10 minutes. After the addition of excess of hot benzene, the silver salts were removed by filtration, the benzene distilled in a vacuum, the residue dissolved in acetone, and the solvent evaporated. A solution of the residual syrup in acetone (100 c.c.) was poured into water (500 c.c.), and the hepta-acetate triturated until it became semi-solid; it then crystallised from warm alcohol, forming plates (2 g.), m. p. 213°.

Hepta-acetyl primeverose (5 g.) gradually dissolved in a mixture of acetic acid saturated with hydrogen bromide (12 c.c.) and acetic anhydride (5 c.c.), and after having been kept at room temperature for 2 hours, the solution was diluted with chloroform (150 c.c.) and poured into ice-water (400 g.). The chloroform layer was separated, washed with ice-water (4 × 200 c.c.), and dried with calcium chloride, the greater part of the solvent was removed in a vacuum, and hexa-acetyl primeverosidyl bromide precipitated with an excess of light petroleum (40—50°). This compound was obtained as a colourless glass which became friable when exposed to a high vacuum.

A mixture of well-dried bromide (3 g.), resublimed alizarin (1 g.), quinoline (6 c.c.), and silver oxide (1·5 g.) was vigorously agitated and after the mild reaction had ceased (15 minutes) the reaction mixture was kept in a desiccator for 1 hour. A solution of the product in hot acetic acid (50 c.c.) was poured into water (300 c.c.), the solid collected, drained, and extracted with warm acetic acid (50 c.c.), the filtered extract mixed with warm water (500 c.c. at 40—45°), and the yellow precipitate dissolved in hot alcohol. On cooling, an amorphous yellow solid separated, which was repeatedly purified by hot alcohol until a specimen gave a bright cherry-red colour with cold alcoholic sodium hydroxide, indicating the absence of alizarin. Acetylation of this material with pyridine and acetic anhydride gave the *hepta-acetate* of alizarin β-primeveroside, which separated from warm acetic acid–alcohol (1 : 5) as an amorphous pale yellow solid at first, but was finally obtained in pale yellow needles, m. p. 241° (Found: C, 56·1; H, 5·0. $C_{39}H_{40}O_{20}$ requires C, 56·5; H, 4·8%). Mixed with the acetate of ruberythric acid, it melted at 205—210°.

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